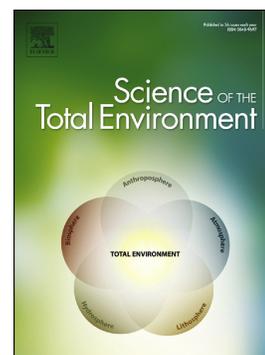


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PII: S0048-9697(23)04417-0

DOI: <https://doi.org/10.1016/j.scitotenv.2023.165794>

Reference: STOTEN 165794

To appear in: *Science of the Total Environment*

Received date: 30 January 2023

Revised date: 22 May 2023

Accepted date: 23 July 2023

Please cite this article as: B.M. Carreira, V. Kolář, E. Chmelová, et al., Bioaccumulation of chemical elements at post-industrial freshwater sites varies predictably between habitats, elements and taxa: A power law approach, *Science of the Total Environment* (2023), <https://doi.org/10.1016/j.scitotenv.2023.165794>

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## Bioaccumulation of chemical elements at post-industrial freshwater sites varies predictably between habitats, elements and taxa: a power law approach

Bruno M. Carreira<sup>a,b,c,\*</sup>, Vojtěch Kolář<sup>a,b</sup>, Eliška Chmelová<sup>b,d</sup>, Jiří Jan<sup>a,b</sup>, Josip Adašević, Andrea Landeira-Dabarca<sup>a,b</sup>, Lucie Vebrová<sup>a</sup>, Martina Poláková<sup>a,f</sup>, Petra Horká<sup>g</sup>, Šárka Otáhalová<sup>b</sup>, Zuzana Musilová<sup>h</sup>, Jakub Borovec<sup>a,b</sup>, Robert Tropek<sup>b,d</sup>, David S. Boukal<sup>a,b</sup>

<sup>a</sup>University of South Bohemia, Faculty of Science, Departments of Ecosystem Biology and Botany, Branišovská 1760, 37005 České Budějovice, Czechia

<sup>b</sup>Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 1160/31, 37005 České Budějovice, Czechia

<sup>c</sup>E3c – Centre for Ecology, Evolution and Environmental Changes, Faculty of Sciences of the University of Lisbon, Edifício C2, Campo Grande, 1749-016 Lisbon, Portugal

<sup>d</sup>Charles University, Faculty of Science, Department of Ecology, Viničná 7, 12844 Prague, Czechia

<sup>e</sup>HAS Den Bosch University of Applied Science, Department of Biology, Animal and Environment, Has Green Academy Po Box 90108, 5200 MA's-Hertogenbosch, The Netherlands

<sup>f</sup>Masaryk University, Faculty of Science, Department of Botany and Zoology, Kotlářská 2, 61137, Brno, Czechia

<sup>g</sup>Charles University, Faculty of Science, Institute of Environmental Studies, Benátská 2, 12801 Prague, Czechia

<sup>h</sup>Charles University, Faculty of Science, Department of Zoology, Viničná 7, 12844 Prague, Czechia

\*Corresponding author: Bruno M. Carreira (bmcarreira@fc.ul.pt)

Bruno M. Carreira: <https://orcid.org/0000-0002-5910-4619>; [bmcarreira@fc.ul.pt](mailto:bmcarreira@fc.ul.pt)

Vojtěch Kolář: <https://orcid.org/0000-0001-6144-317X>; [kolarvojta@seznam.cz](mailto:kolarvojta@seznam.cz)

Eliška Chmelová: <https://orcid.org/0000-0003-3994-2161>; [paddysek@gmail.com](mailto:paddysek@gmail.com)

Jiří Jan: <https://orcid.org/0000-0002-2264-0418>; [jiri.jan@hbu.cas.cz](mailto:jiri.jan@hbu.cas.cz)

Josip Adašević: [josipadasevic@hotmail.com](mailto:josipadasevic@hotmail.com)

Andrea Landeira-Dabarca: <https://orcid.org/0000-0001-8878-8223>; [andrealandab@gmail.com](mailto:andrealandab@gmail.com)

Lucie Vebrová: <https://orcid.org/0000-0002-0819-7245>; [lucka.veb@seznam.cz](mailto:lucka.veb@seznam.cz)

Martina Poláková: <https://orcid.org/0000-0002-0400-4530>; [polakova.23@email.cz](mailto:polakova.23@email.cz)

Petra Horká: <https://orcid.org/0000-0002-5407-7594>; [petra.horka@natur.cuni.cz](mailto:petra.horka@natur.cuni.cz)

Šárka Otáhalová: [sarka.otahalova@upb.cas.cz](mailto:sarka.otahalova@upb.cas.cz)

Zuzana Musilová: <https://orcid.org/0000-0001-8759-8663>; [zuzana.musilova@natur.cuni.cz](mailto:zuzana.musilova@natur.cuni.cz)

Jakub Borovec: <https://orcid.org/0000-0002-1259-8703>; [jakub.borovec@bc.cas.cz](mailto:jakub.borovec@bc.cas.cz)

Robert Tropek: <https://orcid.org/0000-0001-7499-6259>; [robert.tropek@gmail.com](mailto:robert.tropek@gmail.com)

David S. Boukal: <https://orcid.org/0000-0001-8181-7458>; [dboukal@prf.jcu.cz](mailto:dboukal@prf.jcu.cz)

## Abstract

Elevated environmental levels of elements originating from anthropogenic activities threaten natural communities and public health, as these elements can persist and bioaccumulate in the environment. However, their environmental risks and bioaccumulation patterns are often habitat-, species- and element-specific. We studied the bioaccumulation patterns of 11 elements in seven freshwater taxa in post-mining habitats in the Czech Republic, ranging from less polluted mining ponds to highly polluted fly ash lagoons. We found nonlinear, power-law relationships between the environmental and tissue concentrations of the elements, which may explain differences in bioaccumulation factors (BAF) reported in the literature. Tissue concentrations were driven by the environmental concentrations in non-essential elements (Al, As, Co, Cr, Ni, Pb and V), but this dependence was limited in essential elements (Cu, Mn, Se and Zn). Tissue concentrations of most elements were also more closely related to substrate than to water concentrations. Bioaccumulation was habitat specific in eight elements: stronger in mining ponds for Al and Pb, and stronger in fly ash lagoons for As, Cu, Mn, Pb, Se, V and Zn, although the differences were often minor. Bioaccumulation of some elements further increased in mineral-rich localities. Proximity to substrate, rather than trophic level, drove increased bioaccumulation levels across taxa. This highlights the importance of substrate as a pollutant reservoir in standing freshwaters and suggests that benthic taxa, such as molluscs (e.g., *Physella*) and other macroinvertebrates (e.g., *Nepa*), constitute good bioindicators. Despite the higher environmental risks in fly ash lagoons than in mining ponds, the observed ability of freshwater biota to sustain pollution supports the conservation potential of post-industrial sites. The power law approach used here to quantify and disentangle the effects of various bioaccumulation drivers may be helpful in additional contexts, increasing our ability to predict the effects of other contaminants and environmental hazards on biota.

**Keywords:** trace elements, heavy metals, macroinvertebrates, fish, microhabitat, trophic level, coal combustion residues

## 1. Introduction

The extraction and processing of mineral resources associated with rapid industrialization, urbanization, agriculture, and other human activities lead to increased concentrations of heavy metals and other elements naturally occurring in the environment (Ahmed et al., 2019). The accelerated mobilization of these elements and their elevated levels constitute a well acknowledged form of pollution that causes significant environmental degradation (Cherry and Guthrie, 1977; Ali et al., 2019) and has become a major concern worldwide (Islam et al., 2018). These elements persist and accumulate in the environment, posing a serious threat to natural communities and public health (Ali et al., 2019; Xia et al., 2019). This includes bioaccumulation by individual organisms, which can inhibit their growth, suppress oxygen consumption, and impair reproduction and tissue repair (Brázová et al., 2012). However, the severity of these effects and environmental risks are often habitat-, species- and element-specific (Johnson et al., 2017; Llorca and Rainbow, 2005). Large-scale multi-trophic and multi-taxa studies documenting pollution by heavy metals, and other elements, and their bioaccumulation patterns in post-industrial freshwater communities are surprisingly rare (Rahman et al., 2012; Chen et al., 2000; Liu et al., 2022). Although bioaccumulation patterns have been assessed in industry-polluted streams or estuaries (Ahmed et al., 2019; Gavhane et al., 2021), very few studies have examined post-industrial standing freshwater habitats (Pouil et al., 2020; Rowe, 2014). This makes identifying any general trends in bioaccumulation difficult owing to the context dependence detailed below. Moreover, the focus on economically valuable taxa (e.g., fishes; (Ahmed et al., 2019; Gheorghe et al., 2017; Liu et al., 2022) has led to a knowledge gap on invertebrates that may also serve as bioindicators.

Concentrations of elements in individuals are driven by environmental concentrations and bioaccumulation rates (Cain et al., 2004; de Boer et al., 2018). In aquatic environments, bioaccumulation is often quantified by the substrate- and water-based bioaccumulation factor

(BAF), i.e., the ratio between the tissue and environmental concentrations (McGeer et al., 2003; O’Callaghan et al., 2022). Bioaccumulation can differ between habitat types (Fletcher et al., 2020), depending on substrate rugosity (Yu et al., 2012) and chemistry (O’Callaghan et al., 2022), as well as between localities, as demonstrated by DeForest et al. (2007) and McGeer et al. (2003), who found widespread inverse relationships between exposure concentrations and BAF values.

Bioaccumulation can further depend on the microhabitat used by the organism (Culioli et al., 2009; Fletcher et al., 2020). Benthic taxa are often more susceptible (Gundacker, 2000), since sediments can accumulate elements such as As, Cu, Mn, Ni, Se and Zn in high concentrations (Bere et al., 2016; Cherry and Guthrie, 1977), acting as reservoirs that release them back into the water column (di Veroli et al., 2014; Frémion et al., 2016). Concentrations in animal tissues may thus differ substantially between taxa (e.g., benthic versus pelagic), and the differences often relate more to substrate than to water concentrations (e.g., O’Callaghan et al., 2022), implying that substrate-based BAFs should be more reliable predictors of susceptibility to pollution. Bioaccumulation can also differ between species that use different mechanisms to maintain low intracellular concentrations of elements, such as limited uptake and depuration (Fletcher et al., 2022; Tollett et al., 2009). More resilient arthropods can shed excess concentrations during larval moults and maintain low BAF values (e.g., odonates: Stoks et al., 2015; but see Buckland-Nicks et al., 2014), while soft-bodied taxa can be highly sensitive to some elements, e.g., *Cloeon* and *Asellus* to Cd, Cu, and Ni (Milani et al., 2003).

Bioaccumulation also differs between elements, especially between essential and non-essential ones (Andres et al., 2000). BAF values of essential elements (e.g., Cu, Fe, Ni, and Zn) should increase disproportionately at low environmental concentrations as individuals bioaccumulate them to maintain homeostasis, leading to higher BAF values than those of non-essential elements. This is expected to yield non-linear, element-specific relationships between

environmental and tissue concentrations. Power laws have been used to describe such relationships across many biological phenomena (West and Brown, 2005), and can provide a general tool to describe the relationship between tissue and environmental concentrations, as well as to identify the main drivers of its variation. Interestingly, such analyses of bioaccumulation patterns in aquatic ecosystems are virtually absent, but see a recent exception (Atiaga et al., 2021).

To fill these knowledge gaps, we applied the power law approach to study the bioaccumulation of 11 elements in 20 localities in the Czech Republic and identify potential bioindicator species. We quantified the habitat and taxon dependence of bioaccumulation in seven animal taxa, including fish and macroinvertebrates, over large pollution gradients in post-industrial standing waters – mining ponds and fly ash lagoons. Due to the heavy industrialisation in the second half of the 20<sup>th</sup> century, these habitats have become common in many anthropogenic landscapes and can harbour threatened biodiversity (e.g., Řehouňková et al., 2016; Tropek et al., 2013; Kolar et al., this issue), despite the challenging environmental conditions, especially in the more polluted fly ash lagoons (Cherry and Guthrie, 1977; Rowe et al., 2002; Chmelová et al., 2021). They also provide a unique opportunity to investigate bioaccumulation in aquatic communities along steep environmental gradients (Fletcher et al., 2017; Rowe, 2014).

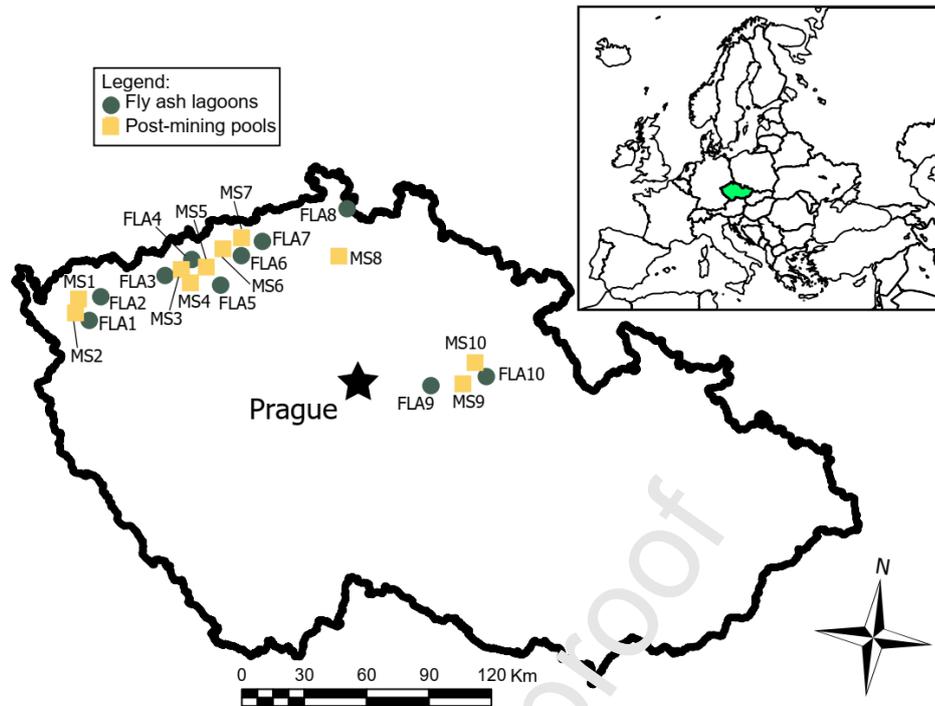
We expected higher environmental concentrations of elements in fly ash lagoons than in mining ponds (Izquierdo and Querol, 2012; Schwartz et al., 2016). Based on the evidence summarized above, we predicted: (1) higher element concentrations in animal tissues in fly ash lagoons than in mining ponds; (2) higher BAF values (i.e., the ratios between tissue and environmental concentrations) in fly ash lagoons than in mining ponds after correcting for the environmental concentration (Fletcher et al., 2020; Yu et al., 2012); (3) better correspondence of tissue concentrations to substrate than to water concentrations (O’Callaghan et al., 2022); (4)

constant or decreasing BAF values at higher environmental concentrations (DeForest et al., 2007), with the latter pattern especially pronounced in essential elements (Andres et al., 2000); and (5) taxon-specific BAF values (e.g., Erasmus et al., 2020) that remain constant or increase with trophic level (Pastorino et al., 2020, 2019); but see Culioli et al., 2009; Hans et al., 2011) and are higher in benthic than in pelagic taxa (Fletcher et al., 2020; Gundacker, 2000; Pastorino et al., 2020; Pouil et al., 2020), making benthic or predatory taxa better potential bioindicators of pollution.

## 2. Materials and methods

### 2.1 Study sites

We studied the bioaccumulation of elements in two types of post-industrial freshwater habitats created artificially in recent decades – 10 fly ash lagoons and 10 mining ponds scattered throughout north-western and eastern Bohemia, the Czech Republic (Fig. 1). The fly ash lagoons are remnants of an originally vast system of sedimentation lagoons, created for the deposition of mineral residues from lignite combustion for energy production in thermal power plants (Kuzmick et al., 2007). Both habitat types share finely grained substrate (fly ash, sand, or clay), low amount of organic nutrients, and minimal agricultural pollution (eutrophication and pesticides; Chmelová et al., 2021). However, the environmental levels of elements in the mineral residues of coal combustion is supposedly much higher than in mining residues (Cherry and Guthrie, 1977; Rowe et al., 2002). The process of sand and clay extraction has little impact on the bioavailability of elements that typically reflects that of the parent bedrock, while the chemical processes during lignite combustion can greatly increase the bioavailability of elements (e.g., As and Se) due to their high mobilization potential at neutral to alkaline pH values, common in fly ash lagoons (Izquierdo and Querol, 2012; Schwartz et al., 2016).



**Figure 1.** Distribution of the 20 post-industrial sites from north-western and eastern Bohemia, Czech Republic, included in the study. Olive grey circles = fly ash lagoons ( $N = 10$ ), yellow-filled squares = post-mining ponds ( $N = 10$ ). See Table S1 for details.

Even though element concentrations rarely reached potentially harmful levels in a recent survey of fly ash lagoons (Chmelová et al., 2021), over 50% of those localities exceeded Czech Republic's national limits for As, Cu, V, or Zn. The sampled fly ash lagoons (surface area: range, 0.1–6.6 ha, mean  $\pm$  SD,  $2.6 \pm 1.8$  ha; Table S1) were chosen based on an extensive survey covering all the localities with remnants of non-reclaimed fly ash deposits in Bohemia, north-western and eastern Czech Republic (Chmelová et al., 2021). The sampled mining ponds (surface area: 0.9–21.7 ha,  $8.1 \pm 5.5$  ha) were chosen from nearby abandoned sand or caoline mines, with one locality originating in a spoil heap after lignite mining and two originating from

mine subsidences, to be as similar as possible to the fly ash lagoons in terms of size, successional stage, and habitat characteristics (Table S1).

### 2.2 Substrate and water sampling

We visited each locality in the summer and autumn of 2018 and in the spring of 2019. During each visit, we collected a 0.125L core of undisturbed sediment (5cm surface layer) at three sites within each locality (except MS9, that partially dried out), to cover possible spatial variation in element concentrations. The cores were collected ca. 1m from the shore in open water without macrophytes or organic detritus. We also took three 1L water samples, mixed them in a clean bucket, and collected a 0.125L mixed sample per locality. The substrate and water samples were stored at -18°C before analysis. At the three sites within each locality (Table S1), we recorded water conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ) and water pH using a portable YSI multimeter (type 556 MPS, YSI Environmental, USA) and sediment pH using a portable Eutech multimeter (type pH 5+, Eutech Instruments Pte Ltd., USA), attached to an ORION electrode (type 9103 BNWP, ORION, USA).

### 2.3 Focal taxa sampling

We analysed bioaccumulation in seven taxa of aquatic macroinvertebrates and fish that differed in trophic level and microhabitat use and were sufficiently common to enable the analyses (Table S2): the snail *Physella acuta* (grazer and detritivore, benthic or found on submerged macrophytes; Horsák et al. 2013), larvae of the dragonfly *Orthetrum cancellatum* (predator, benthic) and *Anax imperator* (predator, phytophilous and usually hiding among vegetation in the water column; Corbet, 1999), adults of the heteropteran *Nepa cinerea* (predator, benthic or hiding among detritus in shallow water), adult diving beetles *Rhantus* spp. (predator, actively hunting near the bottom and in the water column; Burghelea et al., 2011; Hodecek et al., 2016),

and two fish species: roach *Rutilus rutilus* (planktivorous, pelagic), and perch *Perca fluviatilis* (small individuals are planktivorous, but become piscivorous later, pelagic; Kottelat & Freyhof, 2007). This selection carried an unavoidable bias as *Physella* is the only non-predatory taxon that simultaneously has the closest association with the substrate. Nevertheless, other herbivore species were not abundant enough across the study sites to be included in the analyses.

All macroinvertebrates were collected by sweeping with a kitchen strainer (19 cm in diameter; 1mm mesh) and by baited traps (Kolář et al., 2017; Kolar and Boukal, 2020), while fish were caught by electrofishing with a battery Grassl electrofisher (Hans Grassl GmbH) along the littoral zone at each locality. We refer to the taxa by their genus name and treat *Perca*, *Rutilus*, *Rhantus*, and *Anax* as ‘pelagic’, and *Orthetrum*, *Nepa*, and *Physella* as ‘benthic’.

#### 2.4 Analysis of chemical elements

We focused on 11 chemical elements (Al, As, Co, Cr, Cu, Mn, Ni, Pb, Se, V and Zn) that pose high ecotoxicological risks in aquatic deposits of lignite combustion residues (Rowe, 2014; Rowe et al., 2002), and can have pervasive effects on habitats and biota (*sensu* Järup, 2003). Hg and Cd were not included in the analyses, because Hg is almost completely volatilized during coal combustion (Otero Rey et al., 2003), and Cd concentrations in the samples were too close to the detection limit (Chmelová et al., 2021). In addition to these 11 elements, we also quantified the concentrations of Ca and Mg in substrate ( $\text{mg.g}^{-1}$ ) and water ( $\text{mg.L}^{-1}$ ) and concentrations of dissolved organic carbon (DOC,  $\text{mg.L}^{-1}$ ) to assess their potential effect on bioaccumulation patterns (MacMillan et al., 2019; Rüdél et al., 2015). DOC concentrations in water were determined by elemental analysis on TOC-L analyser (Shimadzu, Japan) using high-temperature (680 °C) catalytic oxidation method with an infrared gas analyser (NDIR), after filtering the samples through a 0.45 mm glass fibre filters (MN-GF5, Germany).

Sediment samples were freeze-dried, gently ground with mortar and pestle, sieved through a 2 mm mesh to exclude stones and larger organic debris, ground to dust in a mixer mill, mineralized in duplicates with nitric and perchloric acid for 12 h (Kopáček et al., 2001). Water samples for chemistry analyses were filtered through 0.4  $\mu\text{m}$  pore size glass-fibre filters (MN-5, Macherey Nagel), acidified with the addition of 1% concentrated suprapur nitric acid. Both substrate and water samples were analysed by an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 8800 ICP-QQQ, Agilent Technologies Inc., Tokyo, Japan) to determine the total concentrations of Ca, Mg and the 11 focal chemical elements. We used average values of pH and DOC and average substrate and water concentrations of all elements from each locality in the data analyses.

Animal samples consisted of whole-body tissue for all taxa except for *Physella*, in which the shell was removed before the analysis. The samples ( $N = 362$ ) were frozen and freeze-dried in a Christ ALPHA1-2 LD plus freeze-dryer (Osterode, Germany). The dried material was weighed, ground to dust using a laboratory mixer mill (MM 200, Retsch, Germany), homogenized, and a subsample was mineralized with nitric (115°C) and perchloric acid (170°C) for 12 hours (Kopáček et al., 2001). Subsequently, water was added and the total concentrations of the 11 focal elements in the samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 8800 ICP-QQQ, Agilent Technologies Inc., Japan).

## 2.5 Data analyses

### 2.5.1 Environmental concentrations

We first compared concentrations  $E_{X,e}$  of all elements  $X$  in the substrate and water samples ( $e = s$  for substrate and  $e = w$  for water), using generalized linear mixed models (GLMMs) with Gamma distribution and a log-link function, to determine if the environmental concentrations differed between elements and the two post-industrial freshwater types. Following a model

selection approach (Burnham & Anderson 2002), we built five candidate models for each sample category (Table S3) and included locality as random intercept to account for the multiplicity of elements measured at each locality. We compared the models using the corrected Akaike Information Criterion ( $AIC_c$ ) to identify the most parsimonious model and other plausible models (with  $\Delta AIC_c \leq 2$ ).

We also ran two principal components analyses (PCA) using the concentration of the 11 elements in the substrate and in the water. This allowed us to identify the elements driving the variation within each habitat type, to assess the differentiation among the localities, and to determine if the variation in the environmental concentrations was similar in the substrate and water. Finally, we performed a redundancy analysis (RDA) using substrate and water pH, DOC concentration, and substrate and water concentrations of Ca and Mg (the main drivers of water conductivity) to test potential differences in the environmental conditions of the two habitat types that could affect bioaccumulation patterns (999 permutations). The analyses were performed in Canoco 5 (Ter Braak & Šmilauer, 2012) after transforming (as  $\log_{10}(x+1)$ ) and centring the response variables.

### 2.5.2 International legal limits

We collated available data on the limit values of As, Cr, Cu, Pb, Se and Zn permitted in fish and fishery products for human consumption in the different countries (European Commission, 2006; Nauen, 1983). These data were used to establish the range of legal maximum limits, to which we compared the tissue concentrations found in the focal taxa.

### 2.5.3 Bioaccumulation patterns

Preliminary inspection of the data showed that both environmental and tissue concentrations varied several orders of magnitude within each element, and that a linear relationship between

them, which underlies the calculation of BAF (Goodyear and McNeill, 1999; Mountouris et al., 2002; Verschoor et al., 2012), did not hold for multiple taxa and elements (DeForest et al., 2007). Unlike Peter et al. (2018), who included parameters describing element concentrations in the environment and in food sources of individuals, we used a more general, power scaling relationship between the concentration  $E_{X,e}$  of the element  $X$  in the environment  $e$  and in the animal tissue  $A_X$

$$A_X \sim R_{X,e} (E_{X,e} / \bar{E}_{X,e})^{b_{X,e}} \quad (1a)$$

that is,

$$\ln(A_X) \sim r_{X,e} + b_{X,e} \ln(E_{X,e} / \bar{E}_{X,e}) \quad (1b)$$

where  $r_{X,e} = \ln(R_{X,e})$  is the logarithm of the expected tissue concentration  $R_{X,e}$  at the median concentration  $\bar{E}_{X,e}$  of element  $X$  in environment  $e$  (hereafter ‘BAF intercept’) and  $b_{X,e}$  is the slope of the linearized relationship Eq. 1b on the log-log scale (hereafter ‘BAF slope’). Instead of focusing on the BAF intercepts, we calculated and visualized the predicted values of  $R_{X,e}$  divided by  $\bar{E}_{X,e}$  (hereafter ‘expected BAF values’). Values of  $\bar{E}_{X,e}$  were typically near the maximum environmental concentration in the mining ponds and the minimum value in the fly ash lagoons, or within the overlapping range of concentrations in both types of freshwater bodies (compare Tables S3 and S4).

We used generalized linear mixed models (GLMMs) with Gamma distribution and log-link function to fit Eq. 1b to the data. Zero concentrations in specimen tissues ( $N = 3$ , all Se in natural sites) were replaced by  $10^{-4} \mu\text{g}\cdot\text{g}^{-1}$  to satisfy model assumptions. Water concentration data were converted from  $\mu\text{g}\cdot\text{L}^{-1}$  to  $\mu\text{g}\cdot\text{g}^{-1}$  assuming the density of  $998.2 \text{ g}\cdot\text{L}^{-1}$  at  $20^\circ\text{C}$  to match the units of all concentrations. Variation in the environmental conditions of the different localities was accommodated in the analyses by including locality as a random intercept in all models. Following a model selection approach as above, we compared 18 candidate models for each element by combining nine models for the dependence on the concentrations in each

environment (Table 1). In brief, we assumed that the BAF intercept in each environment  $e$  could be either constant ( $r_{X,e} = r_{X,e}^*$ ), taxon-specific ( $r_{X,e} = r_{X,e}^* + r_{X,e}(\text{Taxon})$ ) or taxon- and habitat-specific ( $r_{X,e} = r_{X,e}^* + r_{X,e}(\text{Taxon}) + r_{X,e}(\text{Habitat})$ ). We used the same logic for the BAF slope:  $b_{X,e}$  could be either constant ( $b_{X,e} = b_{X,e}^*$ ), taxon-specific ( $b_{X,e} = b_{X,e}^* + b_{X,e}(\text{Taxon})$ ) or taxon- and habitat-specific ( $b_{X,e} = b_{X,e}^* + b_{X,e}(\text{Taxon}) + b_{X,e}(\text{Habitat})$ ). Including a separate model where  $b = 0$ , i.e., no dependence of tissue concentrations on the environmental concentration, would have been desirable. However, this approach would have yielded too many models, and we deduced the lack of dependence from the  $b$  value estimates as explained below.

We used a convention in which  $r_{X,e}^*$  and  $b_{X,e}^*$  represent the BAF intercept and slope in *Perca* (the top predator at our sites) in mining ponds,  $r_{X,e}(\text{Taxon})$  and  $b_{X,e}(\text{Taxon})$  describe the difference in the respective parameter between the given taxon and *Perca*, and  $r_{X,e}(\text{Fly ash lagoons})$  and  $b_{X,e}(\text{Fly ash lagoons})$  describe the difference in the respective parameter between fly ash lagoons and mining ponds. We did not attempt to replace taxa with traits in the analyses, as in (Peter et al., 2018), because of the low number of taxa. We comment on candidate traits that may help explain the observed patterns in the Results (section 3.2) and Discussion (section 4.4).

**Table 1.** Structure of models linking element concentrations in animal tissues to environmental concentrations in substrate and water (Eq. 1b). Bioaccumulation factor (BAF) intercept  $r_{X,e} =$  logarithm of expected tissue concentration  $R_{X,e}$  at median concentration  $\bar{E}_{X,e}$  of element  $X$  in environment  $e$ ;  $r_{X,e}^* =$  estimate for *Perca* exposed to median environmental concentration in substrate ( $e = s$ ) or in water ( $e = w$ ) in mining ponds;  $b_{X,e} =$  BAF slope;  $b_{X,e}^* =$  BAF slope for *Perca* in mining ponds. Symbols: T = Taxon and H = Habitat; see Materials and methods (section 2.5.3) for further details.

Environment ( $e$ )	Model	BAF intercept	BAF slope
Substrate ( $s$ )	(s1)	$r_{X,s} = r_{X,s}^* + r_{X,s}(\text{T}) + r_{X,s}(\text{H})$	$b_{X,s} = b_{X,s}^* + b_{X,s}(\text{T}) + b_{X,s}(\text{H})$
	(s2)	$r_{X,s} = r_{X,s}^* + r_{X,s}(\text{T})$	$b_{X,s} = b_{X,s}^* + b_{X,s}(\text{T}) + b_{X,s}(\text{H})$

	(s3)	$r_{X,s} = r_{X,s}^*$	$b_{X,s} = b_{X,s}^* + b_{X,s} (T) + b_{X,s} (H)$
	(s4)	$r_{X,s} = r_{X,s}^* + r_{X,s} (T) + r_{X,s} (H)$	$b_{X,s} = b_{X,s}^* + b_{X,s} (T)$
	(s5)	$r_{X,s} = r_{X,s}^* + r_{X,s} (T)$	$b_{X,s} = b_{X,s}^* + b_{X,s} (T)$
	(s6)	$r_{X,s} = r_{X,s}^*$	$b_{X,s} = b_{X,s}^* + b_{X,s} (T)$
	(s7)	$r_{X,s} = r_{X,s}^* + r_{X,s} (T) + r_{X,s} (H)$	$b_{X,s} = b_{X,s}^*$
	(s8)	$r_{X,s} = r_{X,s}^* + r_{X,s} (T)$	$b_{X,s} = b_{X,s}^*$
	(s9)	$r_{X,s} = r_{X,s}^*$	$b_{X,s} = b_{X,s}^*$
Water ( <i>w</i> )	(w1)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T) + r_{X,w} (H)$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T) + b_{X,w} (H)$
	(w2)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T)$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T) + b_{X,w} (H)$
	(w3)	$r_{X,w} = r_{X,w}^*$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T) + b_{X,w} (H)$
	(w4)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T) + r_{X,w} (H)$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T)$
	(w5)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T)$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T)$
	(w6)	$r_{X,w} = r_{X,w}^*$	$b_{X,w} = b_{X,w}^* + b_{X,w} (T)$
	(w7)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T) + r_{X,w} (H)$	$b_{X,w} = b_{X,w}^*$
	(w8)	$r_{X,w} = r_{X,w}^* + r_{X,w} (T)$	$b_{X,w} = b_{X,w}^*$
	(w9)	$r_{X,w} = r_{X,w}^*$	$b_{X,w} = b_{X,w}^*$

This approach allowed us to determine the extent to which the 11 elements are bioaccumulated in both types of post-industrial freshwater habitats and across the focal taxa, and whether the bioaccumulation levels for the given combination of element and taxon are more tightly linked to the environmental concentrations in substrate or water. We did not include ‘null’ models with no dependence on environmental concentrations, or models with different intercepts or slopes between habitats but not between taxa. The inadequacy of such models for our data (except the most parsimonious model for Cu) was confirmed by exploring the estimates of BAF intercepts and slopes.

Other plausible models ( $\Delta AIC_c \leq 2$ ) were either simplified (with some predictors dropped) or more complex (with some predictors added) versions of the most parsimonious model for all elements except Se (see Results section 3.2 for details). Because additional predictors unique to the other plausible models were of limited relevance, we report and illustrate only the most parsimonious models (except Se, V and Zn, for which we also illustrate the best substrate-based model).

We extracted two measures from each most parsimonious model that characterize the relationship described by Eq. 1b: the expected BAF value  $R_{X,e}/\bar{E}_{X,e}$  and the BAF slope  $b_{X,e}$ , together with the corresponding 95% confidence intervals based on the fixed effects. This allowed us to compare bioaccumulation in the two habitat types across the different elements, to examine how bioaccumulation ratios change with environmental concentrations, and to identify the most bioaccumulated elements and most sensitive focal taxa. We omitted slope estimates for combinations of taxon and habitat type with very short environmental gradients (maximum environmental concentration less than 2.5 times the minimum concentration).

We deemed any differences in expected BAF values  $R_{X,e}/\bar{E}_{X,e}$  and BAF slopes  $b_{X,e}$  between individual taxa or the environments to be significant when the respective 95% confidence intervals did not overlap. Note that the BAF slope  $b_{X,e} = 1$  describes a linear increase of the element  $X$  concentration in the animal tissue with the environmental concentration  $E_{X,e}$ , which makes bioaccumulation factors commonly described in the literature directly comparable across studies (Arnot and Gobas, 2006). Slope  $b_{X,e} = 0$  corresponds to a constant concentration in the animal tissue irrespective of the environmental concentration. Slopes  $b_{X,e} > 1$  and  $1 > b_{X,e} > 0$  correspond to supra-linear and sub-linear scaling relationships, in which the BAF respectively increases and decreases with the environmental concentration. Sub-linear scaling leads to an inverse relationship between BAF and exposure concentration that is widespread in data pooled across multiple studies (DeForest et al., 2007).

Finally, we assessed the effect of environmental gradients on the bioaccumulation patterns. For this, we used water conductivity, substrate and water pH, DOC or concentrations of Ca and Mg in substrate and water as additional predictors in the most parsimonious model (one variable added at a time) and compared the AICc values of the most parsimonious model and the eight additional models for each element. We consider only the effects of additional predictors that resulted in lower AICc and report them as parameter estimates on the predictor

scale with 95% confidence intervals. Including the environmental conditions had only minor quantitative effects on parameter estimates of the model predictors specified in table 1 and did not change the main results. Therefore, we report parameter estimates only for the most parsimonious models without the added environmental predictors, allowing for a direct comparison of the parameters between elements.

All univariate models were analysed in R version 4.1.2 (R Core Team, 2022) using the “glmmTMB” (Brooks et al., 2017) and “bbmle” (Bolker & R Development Core Team, 2021) packages. We used the package “DHARMA” (Hartig, 2022) to explore model residuals and verify that they do not deviate strongly from the model assumptions or present any residual trends. Model summaries were generated with the package “sjPlot” (Lüdtke, 2021).

### 3. Results

#### 3.1 Differences in concentrations between elements and habitat types

Average substrate concentrations of the elements ranked as  $Al > Mn > V > Zn > (As \sim Cu) > (Cr \sim Ni) > Co > (Pb \sim Se)$  in the fly ash lagoons and as  $Al > Mn > Zn > V > (Cr \sim Ni \sim Pb) > Cu > (As \sim Co) > Se$  in the mining ponds (Tables 2 and S5). Average water concentrations of the elements ranked as  $Mn > As > Al > V > Zn > (Ni \sim Se) > (Co \sim Cu) > Cr > Pb$  in the fly ash lagoons and as  $Zn > As > Al > Ni > V > Cu > Mn > (Cr \sim Se) > (Co \sim Pb)$  in the mining ponds (Tables 2 and S6). Substrate concentrations were always higher than water concentrations, but the water-to-substrate ratio of Al, Co, Pb and V was 5–10 times higher in fly ash lagoons than in mining ponds, indicating a greater water saturation with these elements in fly ash lagoons.

Ranges of environmental concentrations of most elements overlapped in both habitat types, although some were found at markedly higher concentrations in fly ash lagoons than in mining ponds (substrate: As and Se, water: Al, As, Se and V; Table 2). Both substrate and water

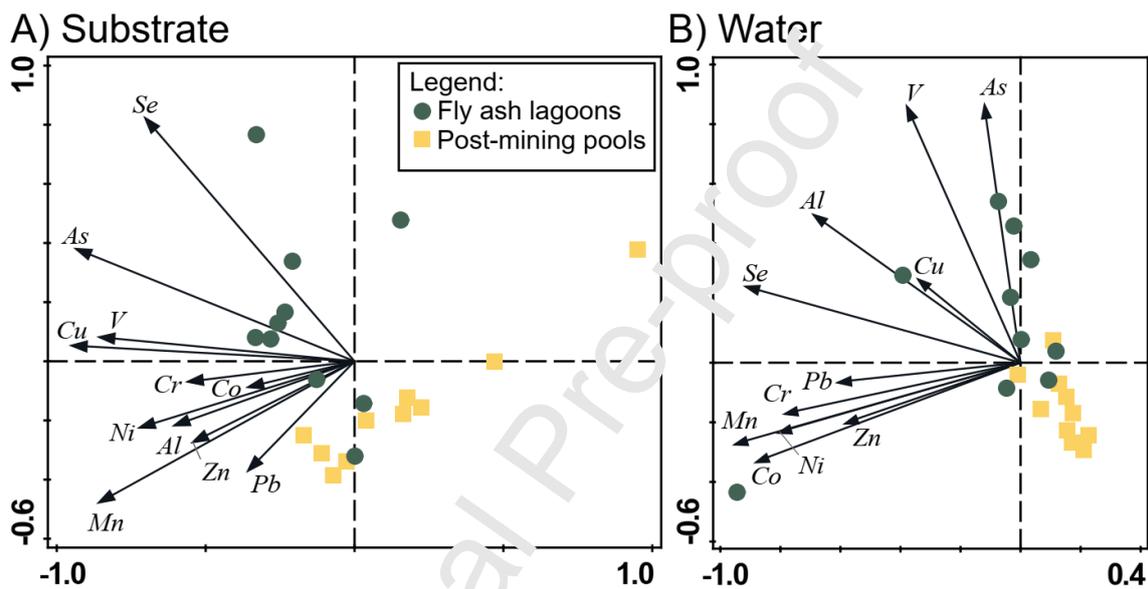
concentrations of the 11 elements were thus best described by the full Gamma GLMM model with element- and habitat-specific values and element-specific differences between the habitat types (Tables S3 and S7).

**Table 2.** Element concentrations (mean  $\pm$  SD, calculated from the mean values at each locality) in the substrate ( $\mu\text{g.g}^{-1}$ ) and water samples ( $\mu\text{g.L}^{-1}$ ) in each habitat type and the Czech national limits in common soils and light sandy soils ( $\text{mg.kg}^{-1} = \mu\text{g.g}^{-1}$ ; Regulation no. 153/2016). See Materials and methods section 2.2 for details. Highlighted values exceed on average the Czech national limits in common soils (marked with \* and boldface) or in light sandy soils (boldface).

Sample	Habitat type	Al	As	Co	Cr	Cu	Mn	Ni	Pb	Se	V	Zn
		Substrate	Mining ponds	29272 $\pm$ 17998	<b>16.5</b> $\pm$ 13.8	16.3 $\pm$ 11.7	33.2 $\pm$ 23.2	28.4 $\pm$ 28.9	393.0 $\pm$ 359.4	35.5 $\pm$ 329.9	32.0 $\pm$ 40.4	0.6 $\pm$ 0.4
	Fly ash lagoons	32372 $\pm$ 12580	* <b>64.4</b> $\pm$ 45.4	19.4 $\pm$ 7.3	52.0 $\pm$ 14.8	* <b>64.0</b> $\pm$ 41.0	703.1 $\pm$ 428.4	* <b>55.6</b> $\pm$ 20.7	12.0 $\pm$ 7.7	13.8 $\pm$ 24.0	* <b>152.7</b> $\pm$ 57.3	81.6 $\pm$ 38.0
Water	Mining ponds	2.6 $\pm$ 1.3	5.9 $\pm$ 8.6	$\pm$ 0.1	$\pm$ 0.1	0.8 $\pm$ 0.6	0.6 $\pm$ 0.9	1.5 $\pm$ 1.0	$\pm$ 0.1	$\pm$ 0.1	1.1 $\pm$ 1.1	$\pm$ 8.6
	Fly ash lagoons	25.4 $\pm$ 30.1	* <b>31.5</b> $\pm$ 41.1	1.2 $\pm$ 3.3	0.3 $\pm$ 0.2	0.9 $\pm$ 0.7	701.0 $\pm$ 2208.1	5.6 $\pm$ 12.3	$\pm$ 0.1	$\pm$ 9.0	18.5 $\pm$ 25.1	$\pm$ 9.3
Limits	Common soil	-	20.0	30.0	90.0	60.0	-	50.0	60.0	-	130	120
	Light sandy soil	-	15.0	20.0	55.0	45.0	-	45.0	55.0	-	120	105

The first 1–2 PCA axes captured most of the explained variation in substrate (axis 1: 65.0%, axis 2: 16.3%; Fig. 2A) and water concentrations (axis 1: 54.5%, axis 2: 20.7%; Fig. 2B). While both habitat types clearly differed in element concentrations in substrate and water, their full ranges overlapped at individual localities (Fig. 2A and 1B). Main gradients in the

substrate concentrations were driven mostly by As, Cu, Mn and V along the first PCA axis and by Se along the second PCA axis. Main gradients in the water concentrations were driven mostly by Co, Cr, Mn, Ni and Se along the first PCA axis and by As and V along the second PCA axis. Locality differentiation within each habitat type was greater in fly ash lagoons, driven mainly by As, Cu, Mn and Se concentrations in the substrate and by Al, As, Se and V concentrations in the water (Fig. 2).



**Fig. 2.** Principal component analyses of the concentrations of the 11 elements in substrate (A) and water (B) samples. Both panels show the first two PCA axes. Olive grey circles = fly ash lagoons, yellow-filled squares = mining ponds. Symbols = individual localities, arrows = passive expressed element concentration gradients.

The two habitat types differed significantly in environmental conditions (RDA: pseudo- $F = 4.2$ ,  $P = 0.023$ ) and habitat type accounted for 14.3% of the adjusted explained variation (Fig. S1). Fly ash lagoons were characterized by lower DOC concentrations and higher concentrations of Ca and Mg, which also meant higher conductivity as compared to mining ponds, while the pH of substrate and water did not differ between the two habitat types (Table

S1; see Kolar et al. in this issue for a more comprehensive analysis of the environmental differences).

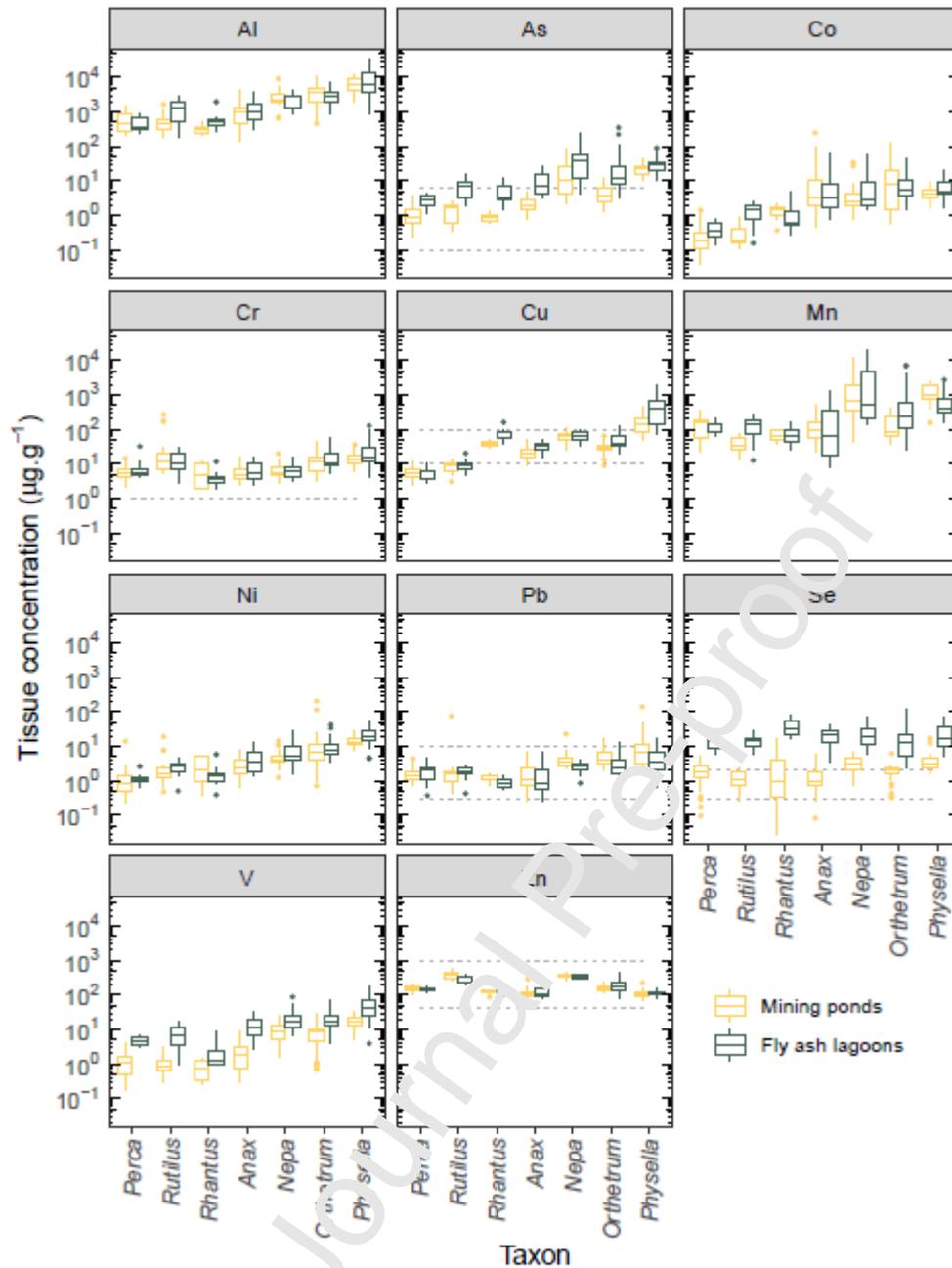
### 3.2 Element concentrations in animal tissues: univariate approach

Animal tissue concentrations varied between elements and taxa but were often similar in both habitat types (Fig. 3, Table S8). We found visibly higher tissue concentrations, with non-overlapping interquartile ranges, in fly ash lagoons than in mining ponds for As (*Perca*, *Rutilus*, *Rhantus*, *Anax* and *Nepa*), Co (*Rutilus*), Cu (*Rhantus*), Mn (*Rutilus*) and Se and V (all taxa; Fig. 3). Average tissue concentrations of individual elements across all taxa ranked in a similar order as the environmental gradient: Al > Mn > Zn > Cu > Se > (As ~ V) > Cr > Ni > Co > Pb in the fly ash lagoons and Al > Mn > Zn > Cu > Cr > As > (Co ~ Ni) > V > Pb in the mining ponds (Fig. 3, Table S8). We observed large variation in the tissue concentrations of the focal taxa, that likely reflected differences in their trophic level, feeding behaviour and microhabitat use. Overall tissue concentrations higher in the benthic taxa (*Orthetrum*, *Nepa* and *Physella*) than in the more pelagic taxa (*Perca*, *Rutilus*, *Rhantus* and *Anax*). For example, tissue concentrations of As in the benthic taxa from the most polluted fly ash lagoons were ca. 10<sup>3</sup> times higher than those in the fish from the least polluted mining ponds (Fig. 3).

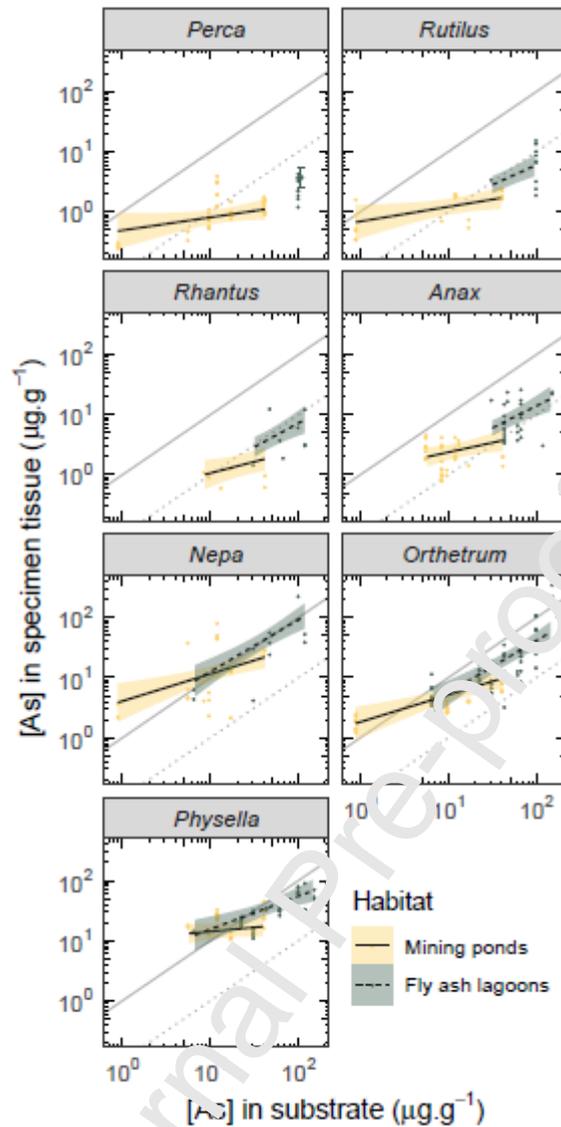
In terms of legal limits in fish and fishery products (Nauen, 1983; EC, 2006), the tissue concentrations of Cu, Pb, and Zn were almost always within or below the range of limits set by individual countries. Tissue concentrations of As were mostly below the limits for the pelagic taxa and mostly above the limit for the benthic taxa; while the tissue concentrations of Cr were all above the single limit value; and the tissue concentrations of Se were mostly within the limits in mining pond samples (except *Nepa* and *Physella*), but always above the limits in fly ash lagoon samples (Fig. 3).

We then analysed how the tissue concentrations depend on the environmental concentrations using Eq. 1b. The most parsimonious GLMM models showed that the tissue concentrations were taxon-specific for each element (Table S9). Habitat type was retained as a predictor of tissue concentrations for eight elements (Al, As, Cu, Mn, Pb, Se, V and Zn), but its effect on the relationship between tissue and environmental concentrations were element-specific (see below Fig. 4 for As, and Fig. S2 for all elements). Tissue concentrations were better described by substrate than by water concentrations (8 out of the 11 elements), although the dependence on environmental concentrations was negligible for Cu (Tables S9 and S10). The most parsimonious models linked tissue concentrations to water concentrations only for Se, V, and Zn (Table S10). However, another plausible model for Se ( $\Delta AICc = 1.4$ ,  $w = 0.161$ ) suggested habitat-dependent tissue concentrations linked to substrate concentrations, with higher tissue concentrations in fly ash lagoons (Table S9).

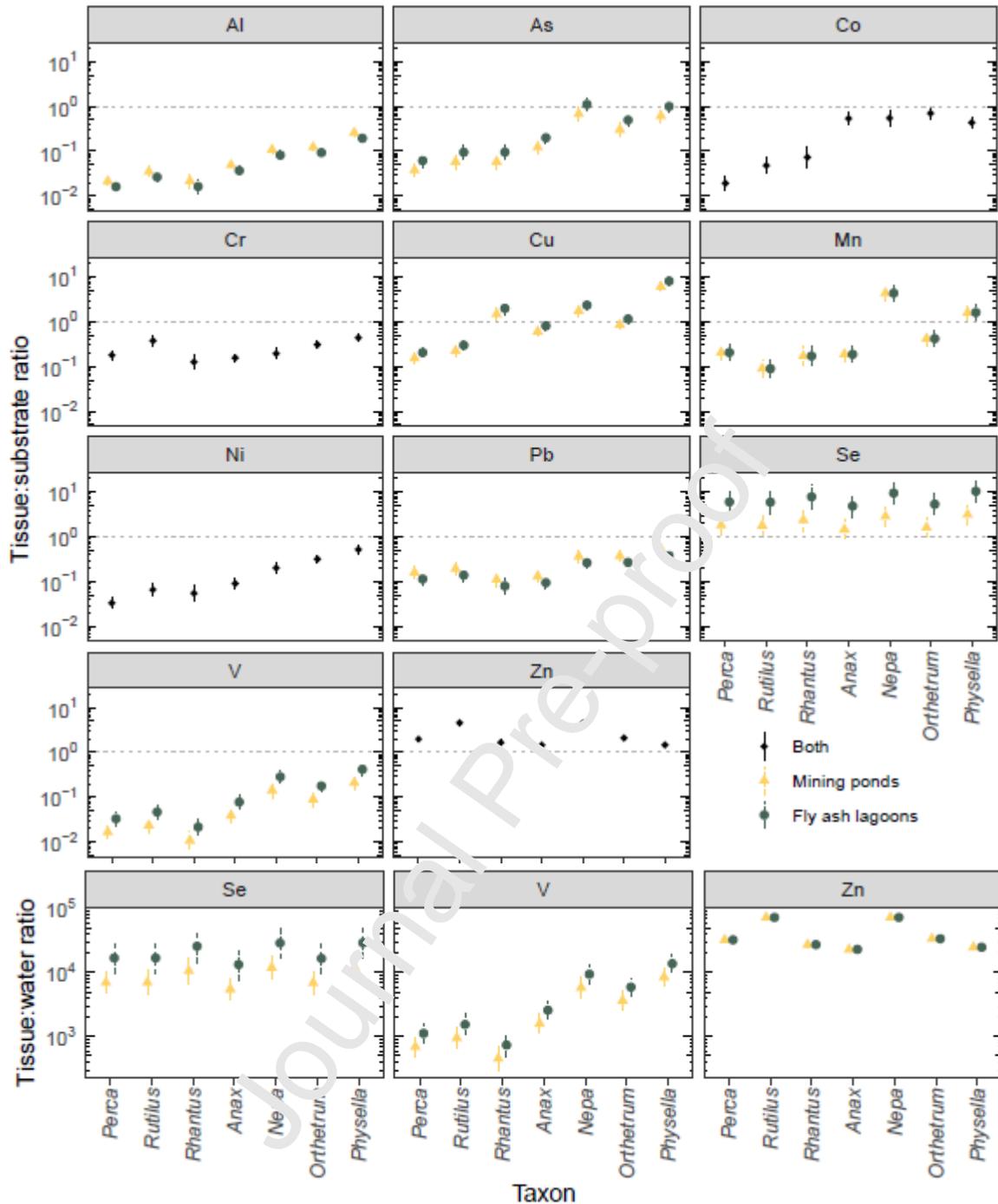
In addition to the environmental concentrations, tissue concentrations of 8 elements (Al, Cr, Cu, Mn, Pb, Se, V and Zn) varied predictably with environmental conditions (Table S11). All else being equal, tissue concentrations of Cu, Pb and Zn increased with higher DOC concentrations. However, tissue concentrations were often higher at mineral-rich localities characterized by high pH or high concentrations of Ca or Mg. Tissue concentrations increased with higher concentrations of Mg in substrate (Al, Pb and V) or in water (Cr, Mn, Pb, V and Zn), Ca in water (Cr, Se and V), pH of substrate (Se) and pH of water (Cu and Se). Surprisingly, tissue concentrations of Mn decreased with high pH values. These additional effects of environmental conditions did not change the main results summarized above, as they caused only minor changes in the estimated effects of environmental concentrations and in the differences between taxa and habitat types.



**Fig. 3.** Observed individual-level animal tissue concentrations of the 11 elements in mining ponds (yellow) and fly ash lagoons (olive grey). Data shown as boxplots with outliers as individual points. Individuals with measured zero Se concentration ( $N = 3$ ) omitted. Dotted horizontal lines illustrate the range of maximum legal limits in fish and fishery products (after Nauen, 1983 and EC, 2006); only one value for Cr).



**Fig. 4.** Prediction of the most parsimonious model linking As concentration in animal tissue to substrate concentration. Black lines with colour band = mean fit  $\pm$  95% CI; points = individual data (mining ponds in yellow, fly ash lagoons in olive grey). Prediction for perch (*Perca*) in fly ash lagoons (larger point with error bar) based on data from a single locality. Grey 45-degree lines show a 1:1 (solid) and 1:10 (dotted) tissue:substrate bioaccumulation ratios.



**Fig. 5.** Expected substrate-based (top four rows) and water-based (bottom row) bioaccumulation factor (BAF) values, calculated as the tissue:environment concentration ratio at the median concentration of element  $X$  in the given environment. Symbols with error bars = estimates with 95% confidence intervals, predicted by the element-specific most parsimonious model and by the best model for substrate-based relationship for Se, V, and Zn (see Materials and methods section 2.5.3 and Table S10 for details). Mining ponds: yellow triangles and lines, fly ash lagoons: olive grey circles and lines; joint value for both habitat types (elements for which the most parsimonious model did not include habitat type as predictor: Co, Cr, Ni, Zn): black diamond and line. Horizontal dashed lines correspond to  $BAF = 1$ .

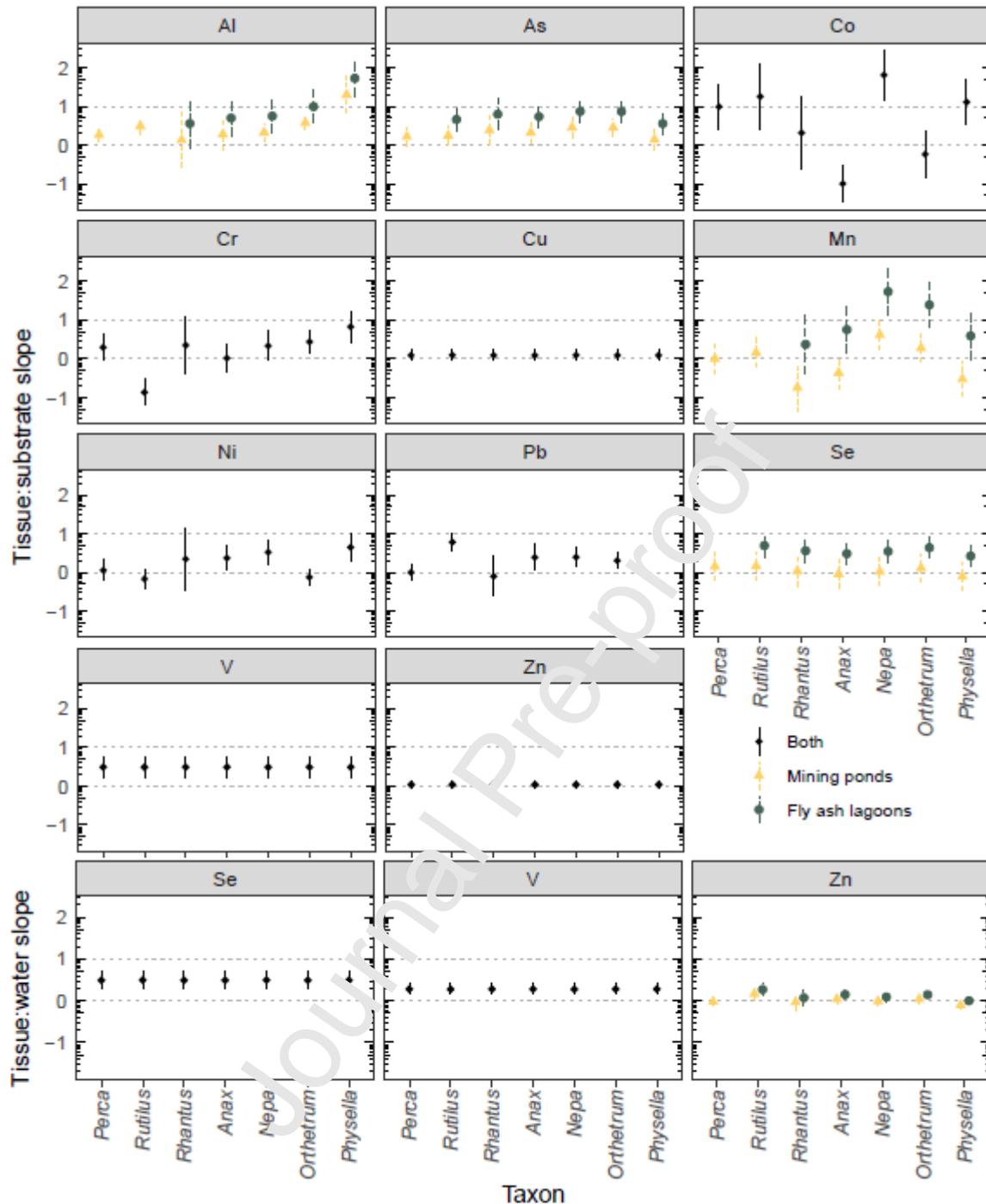
Expected BAF values (i.e., the ratios between the element concentration in the tissue and the environment, calculated at the median environmental concentrations given by Table S4) differed in both habitat types for six elements (Al, As, Cu, Pb, Se, V). We found higher BAF intercepts  $r_{X,e}$  and hence higher expected BAF values in fly ash lagoons than in mining ponds (As, Cu, Se, V), but also the reverse (Al, Pb), with the differences being not significant in Cu and V based on the overlapping 95% CIs (Fig. 5 and Table S10).

Moreover, the expected BAF values were taxon-specific for all elements, although within-habitat differences among taxa in both substrate- and water-based BAF values for Se were not significant (Fig. 5). We observed similar differences in observed, individual BAF values across all elements (Figs. S2 and S3). Expected substrate-based BAF values varied between 0.01–1 for most elements, especially in the pelagic taxa (Fig. 5). However, their values were significantly above 1 for Cu (*Rhantus*, *Nepa* and, especially, *Physella*) and Mn (*Nepa* and *Physella* in mining ponds, Fig. 5), and most or all individual substrate-based BAF values were also higher than 1 for Se and Zn (all taxa; Fig. S3). Expected and observed water-based BAF values were much higher due to the lower element concentrations in the water, with BAF > 1 in nearly all cases (Fig. 5) and individual-level data as high as  $\sim 10^8$ – $10^9$  for Ni (Fig. S4). Expected water-based BAF values of V reflected taxon-specific differences in microhabitat use, with higher values in benthic than in pelagic taxa (Fig. 5).

Although the relationships between the expected BAF values of individual taxa associated with each microhabitat (i.e., pelagic or benthic) varied between elements, common patterns emerged in the predictions of the most parsimonious models. For example, expected BAF values were often lowest or joint lowest in one of the pelagic taxa (*Perca*: Al, As, Co, Cu, Ni; *Perca* or *Rutilus*: Mn; *Rhantus*: Al, Cr, Pb, V), and highest or joint highest in one of the benthic taxa (*Nepa*: As, Mn, Se, Zn; *Orthetrum*: Co; *Physella*: Al, Cr, Cu, Ni, Pb, Se, V; Fig. 5).

BAF slopes (i.e., the exponents  $b_{x,e}$  of the power law relationship linking environmental and tissue concentrations) of the most parsimonious models were neither habitat- nor taxon-specific for three elements (Cu, Se, V), only taxon-specific for four elements (Co, Cr, Ni, Pb), and both taxon- and habitat-specific for four elements (Al, As, Mn, Zn; Figs. 4, 6 and S1, Table S10). In addition, the BAF slopes in the plausible, substrate-based model for Se (Table S9) were both habitat- and taxon-specific. Additionally, the BAF slopes were always steeper in fly ash lagoons than in mining ponds when the slopes differed between the two freshwater bodies, especially for Mn (Fig. 6).

These BAF slopes differed among taxa and elements, but most of the mean predicted values were constrained between 0 and 1 (corresponding, respectively, to constant tissue concentrations that are independent of the environmental concentrations, or sub-linear scaling of tissue concentrations with environmental concentrations; Table S9 and Fig. 6). Although most 95% confidence intervals overlapped with the (0,1) interval in the 11 elements, the values for at least some taxa-by-environment combinations were significantly below 1 (corresponding to sub-linear scaling; Fig. 6). BAF slopes of Cu and Zn were very close to zero, and we also detected significantly negative BAF slopes (i.e., whole 95% confidence interval  $< 0$ , but see Discussion section 4.4 for possible limitations of the data) in *Anax* (Co), *Rutilus* (Cr), *Rhantus* (Mn in mining ponds), and *Physella* (Mn in mining ponds; Fig. 6). Finally, significantly supra-linear scaling (whole 95% confidence interval  $> 1$ ) of the tissue concentrations with the environmental concentration was found in *Nepa* (Co, and Mn in fly ash lagoons) and *Physella* (Al in fly ash lagoons; Fig. 6).



**Fig. 6.** Estimated bioaccumulation factor (BAF) slopes for the tissue:substrate ( $b_{X,s}$ , top four rows) and tissue:water ( $b_{X,w}$ , bottom row) relationships. Symbols with error bars = estimates with 95% confidence intervals, predicted by the element-specific most parsimonious models and by the best model for substrate-based relationship for Se, V, and Zn (see Materials and methods section 2.5.3 and Table S9 for details). Slopes for *Perca* (Al, As, Mn, and Zn) and *Rutilus* (Mn) in fly ash lagoons not shown due to short environmental gradients. Mining ponds: yellow triangles and lines, fly ash lagoons: olive grey circles and lines; joint value for both habitat types (elements for which the most parsimonious model did not include habitat type as

predictor for the BAF slope; substrate: Co, Cr, Cu, Ni, Pb, V, Zn; water: Se, V): black diamonds and solid lines. Dashed horizontal lines mark constant (BAF slope = 0) and linear (BAF slope = 1) relationship between tissue and environmental concentrations; intermediate values correspond to sub-linear scaling.

#### 4. Discussion

We assessed the effects of anthropogenic pollution in post-industrial freshwater habitats, investigating the bioaccumulation patterns of 11 elements in seven animal taxa from two habitat types. The power laws applied to our large dataset provide an insightful overview of the bioaccumulation patterns of the focal taxa along large environmental pollution gradients. Our results suggest that, except for essential elements (especially Cu, Mn, Se, and Zn), bioaccumulation is driven by the environmental concentrations of elements rather than by habitat-specific bioaccumulation patterns or trophic level. Furthermore, tissue concentrations of most elements were better described by substrate concentrations, with proximity to the substrate constituting the main driver of variation in bioaccumulation across taxa. Our findings suggest that many freshwater animals can sustain the elevated environmental concentrations of elements at post-industrial sites and that benthic taxa such as aquatic snails constitute its best bioindicators.

##### 4.1 *Differences between habitat types*

Post-industrial sites may pose environmental risks and public safety issues (Luo et al., 2020; Neupane and Donahoe, 2013) associated with high concentrations of elements (Chmelová et al., 2021; Poláková et al., 2022; Rowe et al., 2002). The large variation in the element concentrations among localities likely reflects differences in the chemical composition of the parent bedrock, extraction, or combustion processes, and different (but unavailable) history of the management of the freshwater bodies. Nevertheless, the differentiation between

the two habitat types was clear in both substrate and water samples (Fig. 2) suggests that fly ash lagoons pose a greater environmental risk than mining ponds (Tables 2 and S5–S7). In fly ash lagoons, the substrate concentrations of As, Mn, and especially Se, were higher than reported recently in a larger survey of 19 localities that included our study sites (Chmelová et al., 2021), and As and V exceeded the limits for common and light sandy agricultural soils, which in mining ponds were only exceeded for Pb (Table 2). In sum, we confirmed our expectation that the environmental concentrations of elements are higher in fly ash lagoons than in mining ponds, although only for some and not all elements. For instance, the mean environmental concentrations of Pb and Zn in the substrate were higher in mining ponds than in fly ash lagoons.

Concentrations of individual elements in tissue samples of a given taxon were often comparable in both habitat types. The concentrations were never higher in mining ponds, while higher values in fly ash lagoons were restricted to seven elements and one or a few taxa (Al, Co, Cu, Mn) or most or all taxa (As, Se, V; Fig. 3). Of these elements, only the tissue concentrations of As (benthic taxa) and Se (all taxa) were above the range of legal maximum limits permitted in fish and fishery products (Nauen, 1983; EC, 2006). Overall, the results partially supported our prediction (1) that concentrations in animal tissues should be higher in fly ash lagoons. This corroborates previous studies showing that pollution with As and Se, and to a lesser extent other elements (Al, Co, Cu, Mn, V), may be of concern (Izquierdo and Querol, 2012; Schwartz et al., 2016). Notwithstanding the greater environmental risk in fly ash lagoons, both habitat types represent early successional stages that can provide refuge to numerous threatened species in anthropogenic European landscapes (Kolar et al., 2021a,b). Although this potential for biodiversity conservation is better documented in the terrestrial domain (Tropek et al., 2014, 2013), the same may hold for the freshwater counterparts, as supported by previous

studies in mining ponds (Kolar et al., 2021; Poláková et al., 2022) and fly ash lagoons (Kolář et al., 2015; Sroka et al., 2022; Kolar et al., this issue).

Contrary to our prediction (2), bioaccumulation was not always higher in fly ash lagoons than in mining ponds for all elements. The most parsimonious models included habitat either in the BAF intercept or in the BAF slope only for eight elements (Al, As, Cu, Mn, Pb, Se, V, Zn). More importantly, the estimated differences were element-specific and often minor (Figs. 4–6 and S2). Nevertheless, we identified strong trends in the estimated substrate-based (Al, As, Mn, Se) and water-based BAF slopes (Zn) consistent with a faster increase in bioaccumulation along the environmental gradient in fly ash lagoons than in mining ponds (Fig. 6). Overall, our results imply that the bioaccumulation of elements depends primarily on the environmental concentrations, while the element-specific habitat effects found in our study corroborate previous findings (Fletcher et al., 2020; Yu et al., 2012). Thus, our results suggest that, as the environmental concentrations increase, aquatic biota may be at a greater risk in fly ash lagoons than in mining ponds, raising motive for concern and awareness to the need of close monitoring of element concentrations in fly ash lagoons.

#### 4.2 Dependence on substrate and water concentrations

Concentrations in animal tissues were more closely related to substrate than water concentrations in most elements as in O’Callaghan et al. (2022) and mostly in line with our prediction (3). Interestingly, water was a better predictor of the tissue concentration for Se, V, and Zn, in which the tissue concentrations did not vary much among taxa and did not depend strongly on the environmental concentrations, i.e., the BAF slope was close to 0 or at least consistently less than 1.

Water-based BAFs may be reliable predictors of the susceptibility to other elements, at least in some of the pelagic taxa analysed in our study (*Perca*, *Rutilus*, *Rhantus* and *Anax*), as the

dependence of bioaccumulation on environmental concentrations can differ between taxa according to their microhabitat use and substrate proximity (e.g., benthic versus pelagic; Chen et al., 2014; Hilgendag et al., 2022). However, our statistical analyses of bioaccumulation were based on element- rather than taxon-specific models, as we focused on the bioaccumulation patterns of the different elements in the two habitat types. This approach prevented us from analysing bioaccumulation and its dependence on the environmental concentrations in substrate and water on a taxon-by-taxon basis, which could be adopted in future studies with a stronger focus on conservation. Overall, the tight relationship between the tissue and substrate concentrations across all taxa in our study highlights the importance of the substrate as a source of pollution in post-industrial sites.

#### 4.3 Differences in bioaccumulation between elements: expected BAF values and slopes

Tissue concentrations of most elements were lower than in the substrate, and the estimated substrate-based BAF values ranged between 0.01 and 1 (Fig. 5). Several mutually non-exclusive mechanisms can explain this result: physiological mechanisms that limit intake (Ali et al., 2019), shedding through depuration processes (e.g., larval moults; Buckland-Nicks et al., 2014), other sources than the substrate (e.g., intake via food with low tissue concentrations; Liu et al., 2019), and low bioavailability of substrate-bound elements.

Some elements broke this general pattern, with higher tissue than substrate concentrations (expected BAF values  $> 1$ ), for example in Cu (*Rhantus*, *Nepa*, and *Physella*), Mn (*Nepa*, and *Physella* in mining ponds), Se and Zn (all taxa; Fig. 5). The predicted shallow BAF slopes ( $b_{X,e} \approx 0$ ) of these elements, associated with none or very limited dependence on environmental concentrations, are consistent with their status of essential elements, critical for the maintenance of biochemical and physiological functions (Orłowski et al., 2020; Proc et al., 2020). However, while the tissue concentrations of Cu and Zn were within normal limits, the

concentrations of Mn and Se exceeded recommended limits, especially in fly ash lagoons (Hejna et al., 2018; Proc et al., 2020). Furthermore, tissue concentrations of the toxic elements As and Pb were also above the maximum values reported in previous studies (Hejna et al., 2018; Proc et al., 2020). The bioaccumulation of the four latter elements is concerning, especially in the benthic taxa of fly ash lagoons, as recent research suggests that invertebrates may be more sensitive to elevated environmental concentrations than vertebrates, calling for a review of regulatory thresholds (Monchanin et al., 2021).

The observed sublinear scaling of tissue concentrations with environmental concentrations (BAF slope  $0 > b_{X,e} > 1$ ) for most elements was consistent with our prediction (4), but we also found important deviations ( $b_{X,e} < 0$  or  $b_{X,e} > 1$ ). Apart from the near-constant tissue concentrations of Cu and Zn discussed above, the tissue concentrations of Co (*Anax*), Cr (*Rutilus*), Mn (*Rhantus* and *Physella*) and Zn (*Physella*) declined with increasing environmental concentrations ( $b_{X,e} < 0$ ); both patterns were also in line with our prediction (4). However, the tissue concentrations of Al (*Physella*), Co and Mn (*Nepa*) increased faster than the environmental concentrations of these elements ( $b_{X,e} > 1$ ), which contradicted our prediction (4). These results reinforce the element-specific character of bioaccumulation and may reflect physiological processes that actively regulate element concentrations in individuals, e.g., by promoting uptake ( $b_{X,e} > 1$ ) or disproportionate excretion through depuration processes ( $b_{X,e} < 0$ ) at high environmental concentrations (Buckland-Nicks et al., 2014).

Our estimates of BAF values and slopes are based on total element concentrations in substrate and water samples without considering potential limits of their bioavailability, e.g., the extent to which the elements were present in mobile or inert forms. We could have underestimated the ‘true’ BAF values or obtained slightly biased slope estimates, as elements in inert forms are not readily available for uptake and the availability relative to total environmental concentrations can differ between the elements and depend on environmental

conditions (MacMillan et al., 2019; Rüdél et al. 2015). We have partly addressed this issue by examining links between environmental conditions and tissue concentrations, and by using locality as a random intercept in the GLMM models to accommodate the variation in the environmental conditions of the study localities. Our findings are mostly in line with the literature, stating that the bioavailability of the elements for uptake is higher in habitats characterised by low DOC concentrations, or high environmental concentrations of Ca and Mg (MacMillan et al., 2019; Rüdél et al. 2015). Although tissue concentrations of Mn decreased with high pH values, this reflects the leaching behaviour of this element, that like other metals is insoluble under near neutral to alkaline conditions (Azquierdo and Querol, 2012). Nevertheless, our main findings are robust as the effects of environmental conditions had only a minor impact on our conclusions.

#### 4.4 Differences in bioaccumulation between taxa

Bioaccumulation of all elements except Sr varied substantially among taxa in our study. Although similar to the findings of previous studies (Erasmus et al., 2020; Pastorino et al., 2020, 2019), these results only partially agreed with our prediction (5), suggesting that the microhabitat rather than trophic level is a key predictor of bioaccumulation for most elements. The expected BAF values followed the gradient in microhabitat proximity to the substrate (*Physella* > (*Orthetrum* ~ *Nepa*) > *Anax* > *Rhantus* > (*Rutilus* ~ *Perca*)) in some elements (Al, C, Cr, Ni), or reflected more generic differences between pelagic (*Perca*, *Rutilus* and *Rhantus*) and benthic taxa (*Nepa*, *Orthetrum* and *Physella*) in other elements (As, Co, Mn, Pb, V).

These patterns imply that taxa strongly associated to the substrate may constitute good bioindicators of pollution in post-industrial sites. Our results highlight the potential of freshwater snails such as *Physella* as reliable bioindicators of this form of pollution (Coerdacier et al. 2003), due to their ubiquity, low mobility, and inability to shed excess

elements through depuration processes (e.g., during larval moults); but also revealed the potential of the true bug *Nepa* as another candidate for a bioindicator species.

The uptake via trophic transfer can vary with element (Luoma and Rainbow, 2005; Schlekot et al., 2004) and feeding guild (Erasmus et al., 2020; Goodyear and McNeill, 1999; Pastorino et al., 2020), but bioaccumulation is usually assumed to increase with trophic level through biomagnification (Kim and Kim, 2016). However, we found little evidence for this increase except consistent differences in *Physella* (detritivore) and *Perca* (top predator). This lack of congruence between bioaccumulation and trophic level (Harp et al., 2017; Rahman et al., 2012) may arise from taxon-specific microhabitat proximity to the substrate that can mask the effect of trophic level. For example, all taxa surveyed by Erasmus et al. (2020), who reported a relationship between bioaccumulation and trophic levels, were strongly associated with the substrate. This was not the case in our study, which included taxa from different trophic levels with diverse hunting modes and preferred microhabitats. Thus, our results suggest that the dependence on trophic level may manifest primarily in taxa with similar levels of environmental exposure mediated by proximity to the main source of pollution (e.g., substrate).

BAF slopes varied strongly among taxa in some (Al, Co, Cr, Mn, Ni) but not all elements, with marked deviations for some combinations of taxa and elements. Negative BAF slopes ( $b_{X,e} < 0$ ) predicted for *Anax* and *Orthetrum* suggest sensitivity of the odonates to Co and Ni, but also resilience to the bioaccumulation of these elements. On the contrary, the high BAF slopes ( $b_{X,e} > 1$ ) predicted for *Nepa* suggest its susceptibility to the bioaccumulation of Co and Mn. The apparent inconsistency of some patterns in BAF slopes across elements (e.g., *Rutilus* relative to the other taxa) may imply element-specific sensitivity in the focal taxa, but could also reflect the inherent uncertainty associated with relatively short environmental gradients for some elements (Al, Co, Cr, Ni) and taxa (*Rutilus*, *Rhantus*, and *Nepa*), particularly in fly ash

lagoons. Future studies conducted along larger environmental gradients than those available to us could clarify these questions.

#### 4.5 Implications for monitoring and conservation of post-mining freshwater habitats

The presence of taxa from different trophic levels with diverse microhabitat preferences and feeding strategies in both habitat types suggests that freshwater biota can sustain the pollution levels at a wide range of post-industrial sites. The fact that most of the focal taxa were only present at a subset of the 20 study localities mirrors other bioaccumulation studies across large environmental and spatial gradients (e.g., Cain et al., 1992). Species distribution in freshwater bodies depends on many factors beyond pollution levels, especially in fishes due to human action.

Nevertheless, the distribution of the macroinvertebrates in our study may reflect not only taxon-specific ecological niches, but also differential sensitivity to the (sub)lethal effects of elevated levels of elements, which affects individuals indirectly through changes in water chemistry (e.g., conductivity and pH; Eeva and Penttinen, 2009; Gillet and Ponge, 2003) and directly through the toxicity of elements (e.g., As, Cu, Mn, Pb, and Zn; Culioli et al., 2009; Lopez et al., 2016; Yu et al., 2012). That is, over time, pollution can reduce organismal abundance and change community structure, via environmental filtering and elimination of the least tolerant taxa (Fleeger et al., 2003; Rohr et al., 2006, 2016). For example, we found *Nepa* at nine mining ponds but only at two fly ash lagoons in our study. Thus, future studies should examine potential relationships between bioaccumulation of the different elements and the presence of the focal taxa in habitat types that differ in pollution levels, such as mining ponds and fly ash lagoons.

## 5. Conclusions

Despite recent advances and attempts to identify general trends in bioaccumulation patterns across taxa, comprehensive and robust assessments are rare (Liu et al., 2019). We showed that analyses based on a power law approach can quantify and disentangle the effects of various drivers of bioaccumulation. This highlights the importance of comprehensive local studies covering many elements and taxa. It also opens new avenues to investigate the complex bioaccumulation patterns of various other contaminants that are of growing importance in the increasingly anthropogenic landscapes (Martínez-Megías and Rico, 2022) and to further our ability to predict the effects of environmental hazards across communities and environments.

Importantly, our study also provided novel insights on the effects of environmental pollution in post-mining freshwater habitats. Differences in tissue concentrations of most elements were driven by substrate rather than water concentrations of the elements, while the effect of habitat type on bioaccumulation patterns was limited in our study. This highlights the importance of the substrate as a reservoir of elements and its polluting potential in standing freshwaters. Furthermore, the microhabitat association of the focal taxa was far more important than their trophic level in determining the bioaccumulation levels. Thus, taxa strongly associated to the substrate such as molluscs (e.g., *Physella*) and other benthic macroinvertebrates (e.g., *Nephelema*) constitute good bioindicators of pollution. Finally, the ability of the freshwater biota to sustain elevated environmental concentrations of elements in post-industrial sites may hold potential for conservation, even if our research showed that fly ash lagoons pose a greater environmental risk than mining ponds.

## Acknowledgments

We thank the owners and administrators of the fly ash deposits for their kind approval of the study and for facilitating the access to the localities. We thank Pavel Potocký and Guillem Casbas-Pinto for their help with the field work and in the collection of specimens. The permit for the collection of fish in all the localities included in this study was issued by the Czech Fishing Association, as officially authorized by the Ministry of Agriculture of the Czech Republic. The permit to handle animals (CZ03061) was issued by the Ministry of Agriculture of the Czech Republic to ZM. This research was funded by the **Czech Science Foundation** (18-15927S), **Czech Academy of Sciences** (Strategy AV21 Land conservation and restoration), and **Foundation for Science and Technology** (CEECIND/02435/2018).

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### Highlights

- Post-mining freshwater habitats polluted by trace elements can harbour rare biota.
- We studied bioaccumulation of 11 elements in seven animal taxa in two habitats.
- We used power laws to link tissue and environmental concentrations of elements.
- Bioaccumulation differed predictably between habitats, elements, and taxa.
- Benthic taxa, such as snails, provide reliable indicators of pollution.

Journal Pre-proof